



## Collective burials among agro-pastoral societies in later Neolithic Germany: perspectives from ancient DNA

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### ABSTRACT

Ancient DNA research has focused on the genetic patterns of the earliest farmers during the European Neolithic, especially with regards to the demographic changes in the transition from hunting and gathering to agriculture. However, genetic data is relatively lacking after this earliest transition period, when societies had fully adapted to new agrarian lifestyles specific to their local environment. During the later central European Neolithic (ca. 3600–2800 cal BC), large-scale collective burials and monumental architecture appeared within the landscape of many agricultural societies. This phenomenon has been argued to represent the emergence of a "collective" identity. With the aim of exploring genetic-based relations among individuals collectively buried, we obtained human skeletal remains of nearly 200 individuals from four later Neolithic collective burial sites in Germany: Calden, Odagsen, Großenrode, and Panker. We successfully reproduced reliable mitochondrial DNA (mtDNA) haplotypes from eight Neolithic individuals, which were assigned to haplogroups H, HV0, and X2. Shared haplotypes observed among individuals within Calden and Odagsen suggest that genetic relations may have shaped the arrangement of the deceased within later Neolithic agricultural groups.

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### 1. Introduction

The transition from hunting and gathering to sedentary agro-pastoralism during the European Neolithic (ca. 6500–4000 cal BC) has been extensively discussed in the archaeological literature (e.g. Gronenborn and Petrasch, 2010; Hodder, 1990; Price, 2001; Whittle and Cummings, 2007). During the fourth millennium BC, diverse forms of settlements appeared across central Europe, fully adapted to the new lifestyles suited to the local environment (Kristiansen, 1982; Midgley, 1992; Müller, 2001, 2011a). A striking landscape distinguished by large-scale collective burials and monumental architecture appeared in northern Europe around 3500 cal BC, constructed by agriculturalists associated with the Funnel Beaker (*Trichterbecher* or TRB) culture (ca.

4100–2800 cal BC). While the emergence of communal graves may have been due to the increase in population size that made the usage of individual burials inconvenient (Sørensen, 2011), scholars have often argued that the monumental burials and associated rich material culture during this period reflects the emergence of social differentiation or a sense of "collectiveness" (Klassen, 2000; Müller, 2001). In particular, these large-scale burials are suggested to have been a specific product designed by the community (Fleming, 1973; Furholt et al., 2012; Renfrew, 1973), symbolizing territorial and status markers, descent groups, or idealized relations of the society (Furholt and Müller, 2011; Kristiansen, 1984; Sjögren, 1986, 2011). Often used over several generations, it is commonly thought that collective burials consisted of individuals from one community representing the anonymous collectivity of ancestors rather than individual identity, but they may also have contained individuals from different families to strengthen socio-political ties between groups (Andersen, 2011; Fleming, 1973; Laporte, 2011; Midgley, 2010; Müller, 2011b; Parker Pearson, 1999). Individuals collectively buried may be examined by genetic analysis to reveal factors such

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as shared biological kinship or deeper uniparental/ancestral relations that may have influenced the arrangement of the deceased (e.g. Haak et al., 2008; Lacan et al., 2011a; Lee et al., 2012).

Ancient DNA research concerning the European Neolithic has mainly focused on elucidating the demographic patterns during the transition from hunting–gathering to agriculture and genetic affinities between and among early Neolithic farming groups (e.g. Linear Pottery, or LBK for *Linienbandkeramik*, ca. 5500–4900 cal BC), indigenous hunter–gatherers, and contemporary populations, including those from the Near East (Bramanti et al., 2009; Gamba et al., 2012; Haak et al., 2010; Malmström et al., 2009). Recent studies have examined regional genetic patterns from later Neolithic contexts, which show continuity as well as variation of genetic lineages between early Neolithic farmers and modern European populations (Deguilloux et al., 2011; Haak et al., 2008; Lacan et al., 2011a, 2011b; Lee et al., 2012). Specifically, burials organized by kinship/ancestral relations have been documented in several later Neolithic contexts. For example, a burial site consisting of nuclear families and the presence of a dominant paternal lineage among male individuals that were collectively buried have been observed (Lacan et al., 2011b; Masset, 1993; Meyer et al., 2008).

As part of a larger interdisciplinary project concerning early monumentality and social differentiation, we collected human skeletal remains from several Neolithic sites associated with monumental burials. The goal of our study was to investigate genetic relations and lineages represented among individuals collectively interred in large-scale burials during the later Neolithic in Germany.

## 2. Material and methods

### 2.1. Samples

Human skeletal material from 201 individuals buried at four collective burial sites was obtained: Calden ( $n = 59$ ), Odagsen ( $n = 107$ ), Großenrode ( $n = 29$ ), and Panker ( $n = 6$ ). All excavated during 1980–1990, Calden, Odagsen, and Großenrode are located within 60 km from each other in central Germany (Hesse and Lower Saxony) dated to 3400–3000 cal BC, while Panker is located in northern Germany (Schleswig-Holstein) dated to 3100–2800 cal BC (Hirsch, 2011; Raetzl-Fabian, 2000; Rinne, 2003) (Fig. 1). The site of Panker is associated with the TRB and Globular Amphorae, but the three sites from central Germany are located in a region bordering TRB to the north-east and show influences from the TRB and Wartberg, a regional Neolithic group (3600–2700 cal BC) in central Germany (Raetzl-Fabian, 2002). The estimated number of individuals ranged from 15 to 200 at each burial, in which animal remains and pottery were also discovered (Gebhardt and Schröder, 2011; Heege, 1992; Hirsch, 2011; Pasda, 2000; Raetzl-Fabian, 2000; Rinne, 2003).

Since initial excavation, skeletal remains had been stored in dry and cool conditions at the Hessian State Museum (Calden), State Museum of Lower Saxony (Odagsen), City Museum and Archive Einbeck (Großenrode), and Schloss Gottorf (Panker). Osteological information of the individuals was not available for analysis. In order to avoid sampling duplication, teeth and jaw fragments were taken from individual skulls. Previous studies from each site have published radiocarbon dates ranging from 3300 to 2800 cal BC (Heege, 1992; Hirsch, 2011; Raetzl-Fabian, 2000; Rinne, 2003), and

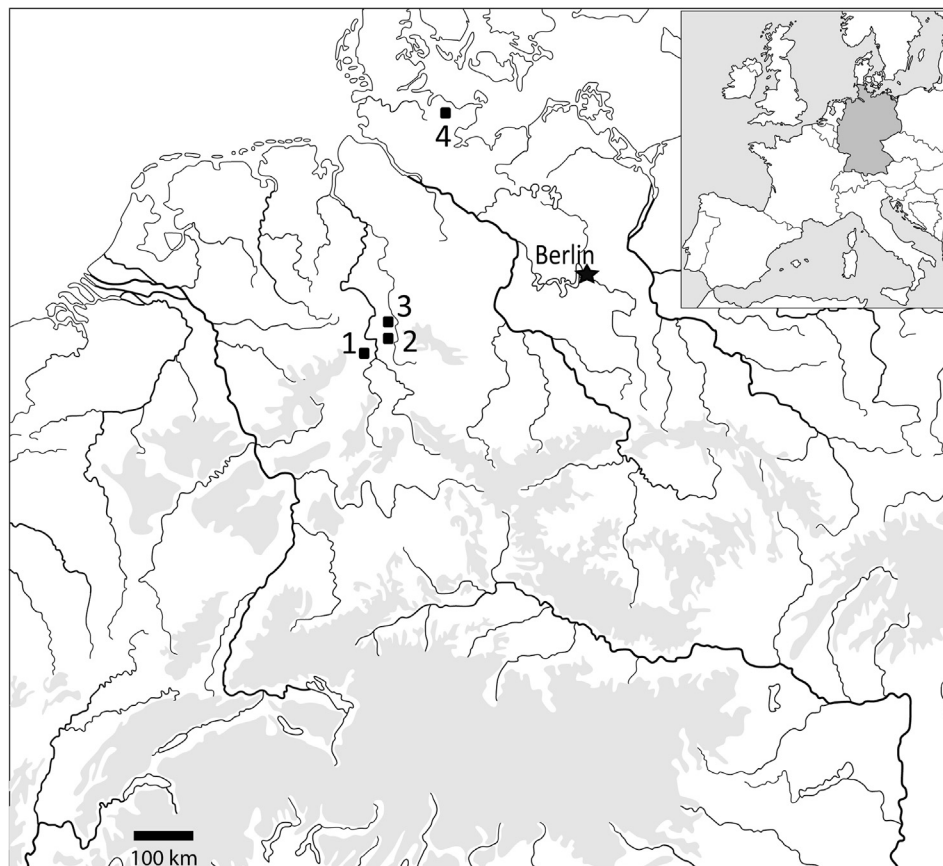


Fig. 1. Location of the collective burial sites examined in this study. Numbers correspond to: 1 – Calden, 2 – Großenrode, 3 – Odagsen, 4 – Panker.

one bone specimen from our study (Calden, KI090; Ref. No. KIA43388) was directly radiocarbon dated (3309–3105 cal BC, 2SD; 3261–3097 cal BC, 1SD), which corresponds with the published and expected dates for the later Neolithic in Germany.

## 2.2. Criteria for ancient DNA authentication

Standards for contamination control and authentication in aDNA research have been recommended and discussed, though there is no formal agreement among scholars (Cooper and Poinar, 2000; Gilbert et al., 2005; Pääbo et al., 2004; Winters et al., 2011; see also discussion in Kemp and Smith, 2010). This study followed the criteria that have been previously described in detail (Lee et al., 2012). All experimental procedures up to PCR (polymerase chain reaction) were carried out in facilities dedicated to aDNA procedures, separate from post-PCR rooms. Disposable coveralls, masks, gloves, and commercially-certified DNA/RNA-free consumables were used, surfaces were bleached prior to experimental procedures and specimens were decontaminated (Kemp and Smith, 2005; Schwark et al., 2012). At least one extraction blank was added for every five samples and at least two negative controls were included for each PCR reaction. Each obtained specimen, two from each individual, was independently extracted and analyzed, and different scientists replicated the procedures at separate times. Results were reproduced by at least two different researchers and all sequences were confirmed by a minimum of two amplified products from each specimen. Only sequences that produced consistent results between different specimens and amplicons were determined reliable. Genetic information from the samples was compared to corresponding data of all lab personnel to identify possible modern contamination and authenticate endogenous genetic data.

## 2.3. Ancient DNA analysis

### 2.3.1. Sample preparation

Preparation of samples followed the same procedures previously described (Lee et al., 2012). Briefly, pulverized specimens were decalcified in EDTA (0.5 M, pH 8.0), followed by an incubation with 20 mg/mL proteinase K and an automated DNA extraction step using the Qiagen BioRobot® EZ1 system.

### 2.3.2. Screening PCR

For an initial screening, a hexaplex PCR, which includes the autosomal polymorphic markers TH01, D3S1358, and D8S1179, the Y-chromosomal marker DYS391, the sex determining marker amelogenin, and a 179-basepair (bp) mitochondrial DNA (mtDNA) fragment, was carried out to quickly determine whether extracts contain amplifiable nuclear DNA and/or mtDNA (Apelt, 2009; see Table A.1 for primer information). Amplification was performed using the Qiagen® Multiplex PCR kit, with 5 µl of DNA extract in a total reaction volume of 12.5 µl. The following conditions were used for PCR: initial denaturation at 95 °C for 15 min, followed by 30 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, then a final step at 60 °C for 30 min. Amplification products were detected on an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems) following manufacturer's protocols.

### 2.3.3. Mitochondrial DNA (mtDNA) analysis

Extracts positive for mtDNA amplification were sequenced for the control region by targeting short fragments ranging 150–180 base pairs (bp) that cover 434 bp of hypervariable region 1 (HV1) in four overlapping regions (nucleotide positions or nps 15975–16429) and 253 bp of hypervariable region 2 in two overlapping regions (nps 00034–00287). Primer sequences and protocols have been previously described (Table 1 and Lee et al., 2012). Haplotypes

**Table 1**

Mitochondrial DNA control region primer sequences used in this study.

Primer	Sequence (5'–3')	Reference
15975F	CTCCACCATTAGCACCCAAAGC	Haak et al. (2010)
16158R	TACTACAGGTGGTCAAGTAT	Lee et al. (2009)
16106F	GCCAGCCACCATGAATATTGT	Lee et al. (2009)
16256R	GCTTTGGAGTTGCAGTTGATGTG	Lee et al. (2009)
16194F	ATGCTTACAAGCAAGTACAGCAA	Lee et al. (2009)
16360R	GAGAAGGGATTGACTGTAATGTG	Fehren-Schmitz et al. (2010)
16268F	CACTAGGATACCAACAACC	Lee et al. (2009)
16429R	GCGGGATATTGATTTACGGA	Fehren-Schmitz et al. (2010)
00034F	GGGAGCTCTCCATGCAITTTGG	Schilz (2006)
00185R	CCTGTAATATTGAACGTAGGTGCGATAA	Schilz (2006)
00120F	CGCAGTATCTGCTTTGATT	Lee et al. (2009)
00287R	TTGTTATGATGCTGTGTGG	Lee et al. (2009)

were determined by identifying mutations in comparison to the revised Cambridge Reference Sequence (rCRS) with Sequencher (Gene Codes, Inc.) (Andrews et al., 1999).

Coding region mutations were typed using a multiplex SNP assay (Heinrich, 2009; Table A.2). Experimental procedures followed previously published protocols (Lee et al., 2012 and Table A.3 for SBE primers).

### 2.3.4. Data analysis

A median-joining network was constructed to examine the phylogeny of the rare maternal lineage haplogroup X2 using Network 4.6 (fluxus-engineering.com; Bandelt et al., 1999). In order to generate a network containing all shortest trees, the reduction threshold was changed to 1 and the post-processing option was utilized. Approximately 400 sequences from contemporary and past populations spanning nps 16040–16390 were compiled from the published literature, excluding Native American X2 haplotypes (Behar et al., 2008; Calafell et al., 1996; Costa et al., 2009; Cui et al., 2010; Derenko et al., 2007; Di Rienzo and Wilson, 1991; Finnilä et al., 2001; Herrnstadt et al., 2002; Malyarchuk et al., 2008; Melchior et al., 2008; Quintana-Murci et al., 2004; Reidla et al., 2003; Richards et al., 2000; Salihović et al., 2011; Shlush et al., 2008; Tömöry et al., 2007; Yao et al., 2004).

## 3. Results and discussion

### 3.1. Amplification success

In the initial screening PCR, partial nuclear DNA profiles were obtained for 31 (15.4%) samples out of 201 individuals (Table 2). The screening results indicate extracts did not contain sufficient amplifiable nuclear DNA for any meaningful evaluation on familial relations. Also based on the screening PCR, 46 samples (22.8%) showed signs of mtDNA amplification. Upon further evaluation, we reproduced reliable mtDNA control region sequences for eight individuals from Panker ( $n = 1$ ), Calden ( $n = 4$ ), and Odagsen ( $n = 3$ ), assigned to haplogroups H1, H2, HV0, and X2 (Table 3). Haplogroups for six individuals were confirmed independently by

**Table 2**

Results of the screening hexaplex PCR.

Site	N	Hexaplex PCR	
		Nuclear <sup>a</sup>	mtDNA
Calden	59	11	13
Odagsen	107	14	21
Großenrode	29	5	6
Panker	6	1	6
<b>Total</b>	<b>201</b>	<b>31 (15.4%)</b>	<b>46 (22.8%)</b>

<sup>a</sup> Partial amplification.

**Table 3**  
MtDNA haplotypes identified in this study and haplogroup assignments.

ID	Site	16042	16179	16189	16223	16255	16263	16278	16297	16298	16362	72	73	153	195	225	263	HG <sup>a</sup>
rCRS		G	C	T	C	G	T	C	T	T	T	T	A	A	T	G	A	
KI056	Panker	—	—	—	—	—	C	—	—	—	—	—	—	—	—	—	G	H1
KI065	Calden	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	H2
KI090	Calden	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	H2
KI116	Calden	—	—	—	—	—	—	—	—	C	—	C	—	—	—	—	G	HV0
KI125	Calden	A	T	C	T	A	—	T	C	—	C	—	G	G	C	A	G	X2
KI164	Odagsen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	H2
KI168	Odagsen	—	—	—	—	—	—	—	—	C	—	C	—	—	—	—	G	HV0
KI186	Odagsen	—	—	—	—	—	—	—	—	C	—	C	—	—	—	—	G	HV0

Mutations are identified in comparison to the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999) (nucleotide positions 15975–16429 and 00034–00287).

<sup>a</sup> HG = haplogroup.

typing coding region mtSNPs (Table A.4). We did not obtain SNP results from the remaining two individuals and no reliable mtDNA data was obtained for the skeletal remains from Großenrode. Soil condition of the region from which the material was obtained is known to be poor and some material had been excavated more than ten years ago. The environmental condition at the sites, time since excavation, and the continuous usage of the burials and burial conditions within the passage graves may have contributed to the overall low success rate. Successful sequences from the eight individuals did not show signs of heterogeneity or double peaks, nor match any of the researchers involved in the experimental procedures. Four distinct haplotypes were identified, and shared haplotypes were verified independently to confirm their authenticity by multiple extracts, amplicons, and sequences, as outlined in Section 2.2. The initial results from the Panker individual were included in the archaeological report for the site (Lee et al., 2011).

### 3.2. MtDNA haplogroups

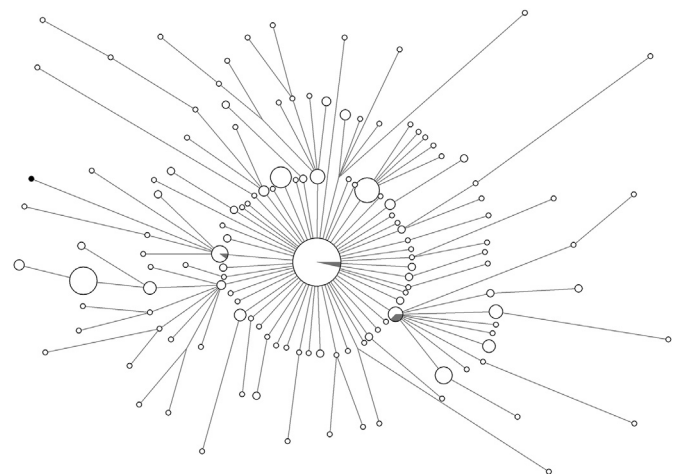
All haplogroups identified in our study, H1, H2, HV0, and X2, have been previously observed among other Neolithic populations (e.g. Deguilloux et al., 2011; Haak et al., 2010; Sampietro et al., 2007), which are also consistent with maternal lineages present among modern Europeans (Achilli et al., 2004; Reidla et al., 2003). Interestingly, we did not identify haplogroup U, which has been documented consistently in various Neolithic and earlier contexts and is thought to be one of the oldest European maternal lineages (e.g. Bramanti et al., 2009; Haak et al., 2010; Lacan et al., 2011b; Lee et al., 2012). Furthermore, haplogroup N1a that is rare in modern Europeans but reported in higher frequencies in early Neolithic groups and also observed at a French megalithic burial chamber was neither observed (Bramanti et al., 2009; Deguilloux et al., 2011; Haak et al., 2010; Palanichamy et al., 2010). Absence of these mtDNA haplogroups in our study may be due to the small sample size.

### 3.3. Characterization of mtDNA lineages

Haplogroups documented in our study can be divided into two major phylogenies, H and X. The rare maternal lineage X2, observed in one individual from Calden (KI125), has an overall frequency of 5% or less among modern Europeans (Richards et al., 2000). Interestingly, haplogroup X2 has not been identified in LBK or earlier Neolithic and Mesolithic groups despite their relatively large sample size ( $n = 59$ ), but documented in late Neolithic (<3000 cal BC) contexts in central Europe (Deguilloux et al., 2011; Haak et al., 2008; Lacan et al., 2011a). In order to further examine this rare maternal lineage, a median-joining network was constructed, in which ancient sequences comprise only ca. 2% of all haplotypes that included the Neolithic X2-haplotypes and three additional haplotypes from a late 10th century medieval cemetery in Hungary

(Tömör et al., 2007) and a Danish Viking site (ca. AD 1000) (Melchior et al., 2008) (Fig. 2). The star-like pattern of haplogroup X2, observed from the large central node with many individuals sharing one haplotype and many branches radiating from this center, is consistent with previous findings (Richards et al., 2000), suggesting a young phylogeny that had rapidly expanded. Strikingly, the X2-haplotype from our study is a derived singleton located at the tip of a branch, while other ancient haplotypes are located at the large central ancestral node or just one mutation derived from the center. Thus based on the current data, either by genetic drift or some demographic event, the X2 lineage from Calden did not continue to the present day.

The origin of haplogroup X2 is still unclear, but studies suggest X2 lineages were introduced into Europe during the Neolithic (Reidla et al., 2003; Richards et al., 2000). On the other hand, haplogroup X1, a subgroup more common in the Near East and North Africa, has been reported in an early Neolithic group from the Iberian peninsula (ca. 5400 cal BC, Gamba et al., 2012). The absence of X2 lineages but presence of X1 in the early farming group in Spain substantiates the scenario in which Neolithic farmers in Europe came from multiple founding populations at different time periods (Deguilloux et al., 2011; Gamba et al., 2012; Lacan et al., 2011b). While genetic drift may have caused the absence of X2 haplotypes among early farmers (e.g. LBK), groups harboring X2 lineages may have migrated into Europe after the early Neolithic into the region, perhaps as small isolated populations in an



**Fig. 2.** Median-joining network of haplogroup X2 comprised of modern and ancient sequences (nps 16040–16390). Each circle represents a haplotype and the size is proportional to the number of individuals that share this haplotype. The length of lines connecting the circles are proportional to the mutational difference between haplotypes. Colors designate haplotypes from the following: gray = ancient populations; white = modern populations; black = this study.



irregular fashion. However, once situated in Europe, it appears X2 lineages were continuously present.

Seven individuals from our study are assigned to haplogroup H and its sister branch HV0. Haplogroup H is the most common maternal lineage in modern European populations (>40%) (Achilli et al., 2004; Richards et al., 2000; Roostalu et al., 2007) and has been found in various Neolithic sites, including the LBK and a late Neolithic Corded Ware group (Haak et al., 2008, 2010; Malmström et al., 2009). Two subhaplogroups of H were identified in our study, H1 (KI056) and H2 (KI065, KI090, KI164). Haplogroup H1 shows a high frequency among modern populations in the Iberian peninsula and H2 in eastern Europe (Pereira et al., 2004, 2005). Three other individuals were assigned to HV0, sharing the same haplotype (KI116, KI168, KI186). Past studies have observed phylogeographic patterns of haplogroups H and HV0 that show a decreasing cline frequency in modern populations radiating from the Iberian peninsula (Achilli et al., 2004; Pereira et al., 2005; Torroni et al., 2001). Scholars have hypothesized that H1 subgroup was derived during a period of refugium in the Iberian peninsula, then afterward expanded as a postglacial dispersal of human populations from southwest to northeast Europe (Pereira et al., 2005). While the overall frequency of haplogroups H and HV0 during the Neolithic remains to be seen, their presence at the collective burials is consistent with previous findings in various Neolithic contexts as well as their prevalence among modern Europeans.

### 3.4. Collective burials during the later Neolithic

The presence of identical haplotypes within Calden (KI065, KI090) and Odagsen (KI168, KI186) implies that at least two individuals with the same maternal ancestry were buried together, though not necessarily simultaneously. As many as 200 individuals were interred at the collective burials, used over several generations spanning hundreds of years (Raetzl-Fabian, 2000; Rinne, 2003). Though we attempted to evaluate as many as 100 individuals from each burial to produce a statistically meaningful sample size, we acknowledge the bias of our small number of reliably reproduced mtDNA data. However, the presence of shared haplotypes within the two burial sites may reflect an intentional practice of burying members from shared descent groups (Hinz, 2007; Sjögren, 1986), perhaps connected by maternal relations. The phenomenon of collectively burying individuals with shared genetic relations also appears at a nearby Bernburg site, Benzingerode, Germany (ca. 3300–3100 cal BC), suggesting this practice was not limited to Wartberg and TRB groups (Meyer et al., 2008).

Shared mtDNA haplotypes between Calden and Odagsen may reflect the interaction between living communities during the later Neolithic documented in the archaeological record that includes influences from Wartberg, TRB and other regional material culture (Raetzl-Fabian, 2002; Rinne, 2003), but may also indicate deeper ancestral relations. It is difficult to elucidate genetic relations between burials based on the shared haplotypes, as the genetic landscape during the later Neolithic is still unclear. However, located at the crossroads of several Neolithic groups, monumental burials may have played an important role in maintaining “collectiveness” based on common identities, genetic, and/or descent relations (Müller, 2011b; Sjögren, 1986).

## 4. Conclusions

Our study examined genetic-based relations among individuals interred in large-scale collective burial sites from Germany during the later Neolithic. We identified four distinct mtDNA haplotypes from eight individuals buried at Calden, Odagsen, and Panker.

Shared haplotypes were observed within Calden and Odagsen, as well as between the two burial sites. The uncommon maternal lineage X2 was observed at Calden, strikingly situated as a derived haplotype in the phylogenetic network. The appearance of X2 after ca. 3000 cal BC may suggest the lineage was introduced into Europe later than others. Haplogroups H and HV0 identified in our study also appear in other Neolithic contexts. Shared haplotypes within Calden and Odagsen suggest maternal relations may have been a factor in collectively burying the deceased during the later Neolithic. Overall, our data highlights the regional genetic landscape associated with monumental burials during the later Neolithic, adding another dimension to the genetic histories of past agrarian societies in Europe.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jas.2012.08.037>.

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